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Optimization of Formulation and Antibacterial Activity of *Citrus limon* Peel Essential Oil and *Eucalyptus globulus* Essential Oil Combination as Gel Hand Sanitizer with The *Simplex Lattice Design* Method

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ABSTRACT

Citrus limon peels essential oil (MACL) and Eucalyptus globulus essential oil (MAEG) have limonene compounds (MACL) and 1,8-cineole (MAEG) which act as antibacterial. This research was conducted to determine the physical properties of the preparation and the inhibition zone of the MACL and MAEG combination formulation as an antibacterial hand sanitizer gel against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa bacteria. The physical properties and antibacterial activity were tested using the Simplex Lattice Design (SLD) method to determine the optimal formula. The results of the physical properties showed that the preparation was in the form of a gel, ivory white, visually homogeneous but under a microscope with 40x and 100x magnification, there were coarse grains; pH value 5.01-5.14; the value of dispersion is 5.54-6.12 cm; the value of adhesion is 6.9-8.1 minutes; and the viscosity value is 170-171.3 dPa.s. Antibacterial activity was tested by the paper disc method. The results showed that F1 100%MACL, F2 100%MAEG, and F3 50%MACL:50%MAEG had antibacterial activity against these bacteria, F1 had inhibition zones, respectively by 9.77 mm, 11.22 mm, and 11.77 mm; F2 had inhibition zones of 10.55 mm, 12.77, and 11.88 mm, and F3 had inhibition zones of 11.66 mm, 11.55 mm, and 10.55 mm. Based on SLD, the optimal formula for hand sanitizer gel is 20% Citrus limon essential oil and 80% Eucalyptus globulus essential oil with pH 5.50 and adhesion 7.20 minutes, zone of inhibition against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa, namely 11.35 mm, 12.22 mm, and 11.44 mm.

***KEYWORDS**: Gel Formulation, Citrus limon, Eucalyptus globulus, Antibacterial, Simplex Lattice Design*

INTRODUCTION

The World Health Organization (2005) stated that reducing nosocomial infections can be done by washing hands using an antiseptic. Besides washing hands with soap, antiseptic liquid or gel, also known as hand sanitizer, can also be used (Rini and Estu, 2018). Alcohol is an active ingredient that is often used as an antibacterial agent in hand sanitizer composition. However, long-term use can dissolve the layer of fat and sebum on the skin (Rohmani & Kuncoro, 2019). To minimize this, natural ingredients such as *Citrus limon* essential oil (CLEO) and *Eucalyptus globulus* essential oil (EGEO) can be used as alternatives for antibacterial active ingredients. Lemon (*Citrus limon*) can act as an antibacterial compound. The compound in the *Citrus limon* essential oil that acts as an antibacterial is Limonene (Friatna et al., 2011; Borgou et al., 2012). Eucalyptus (*Eucalyptus globulus*) is a plant that is also often used as medicine, especially its leaves, which have pharmacological activity because the leaves contain terpenes, porphyrin derivatives, and phenolic compounds. *Eucalyptus globulus* leaves can

act as an anesthetic, antiseptic, deodorant, astringent, disinfectant, expectorant, inhalation, insect repellent, and anthelmintic. The compound that acts as an antibacterial in *Eucalyptus globulus* is 1,8-cineole (Koswandy and Ramadhania, 2016).

Based on research conducted by Hartin & Rini (2019), it was found that lemon juice can inhibit *Staphylococcus aureus* bacteria with an average diameter of 12.1 mm at a concentration of 25% and the resulting inhibition is a slow response. The largest content of lemon peel is essential oil which has properties as a fragrance, perfume ingredient, and adds to the taste of food (Friatna et al., 2011). A compound known as an antibacterial in lemon essential oil, namely limonene, in its effectiveness as an antibacterial in lemons also contains nerol which has a synergistic effect (Borgou et al., 2012). Research conducted by Koswandy & Ramadhania (2016) obtained results, namely *Eucalyptus globulus* essential oil can be used as an antibacterial with concentrations of 1, 2, 5, 7.5, 10, and 20 L to inhibit the growth of *S.aureus* and *E.coli*, a good dose to be used as an antibacterial is a dose of 20µL and the resulting inhibition zone will be bigger and better if the concentration of *Eucalyptus globulus* essential oil is getting bigger. In addition, research conducted by Cahyani & Khoeriyah (2018) related to *Eucalyptus globulus* essential oil in the formulation of cream preparations as an antibacterial *Staphylococcus aureus* ATCC 29213 obtained results, namely *Eucalyptus globulus* essential oil concentration of 10% had antibacterial activity with an average inhibition zone diameter of 1.574 cm.

Therefore, the author conducted further research related the use of *Citrus limon* essential oil and *Eucalyptus globulus* essential oil as an active substance to replace alcohol in hand sanitizers against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* bacteria. The combination of CLEO and EGEO in order to be synergistic as an antibacterial agent, so a larger inhibition zone is formed. Furthermore, *Simplex Lattice Design* (SLD) was used to test the combination of both essential oils to find the best dosage formulation (Ramadhani et al., 2017).

MATERIAL AND METHODS

Material

Citrus limon essential oil and *Eucalyptus globulus* essential oil (CV. Lansida Yogyakarta, DIY). *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* bacteria (Yogyakarta Health and Calibration Laboratory, DIY). Carbopol 940, TEA, methylparaben, glycerin, ethanol 96%, aquadest and nutrient agar (NA) (PT. Brataco). Analytical balance (Iwaki Pyrex®), measuring cup (Iwaki Pyrex®), test tube (Iwaki Pyrex®), volume pipette (Iwaki Pyrex®), beaker (Iwaki Pyrex®), Erlenmeyer (Iwaki Pyrex®), petri dish (Iwaki Pyrex®) dropper, inoculation needles,

tweezers, Bunsen flame, mortar, stirrer, stamper, laminar air flow (LAF), viscometer (Brookfield Ametek®), pH meter (Mettler Toledo®), autoclave (Hirayama® HVE 50 L), incubator (Memmert®), magnetic stirrer (Cimarec®), stirrer bar, adhesion test device

Methods

Formulation Hand Sanitizer Gel

Carbopol 940 was weighed as much as 0.5 grams and sprinkled on 10 ml of distilled water that had been heated. Carbopol 940 which has been sprinkled is stirred rapidly in a mortar until a gel mass is formed and then 0.2 grams of TEA is added. Methyl paraben was weighed as much as 0.2 grams and dissolved in 5 ml 96% ethanol, put in a mortar, stirred until homogeneous. Added glycerin into the mortar, stirred until homogeneous. Take 1 ml of *Citrus limon* peel essential oil then add 10 ml of 96% ethanol and put into formula 1, take 1 ml of *Eucalyptus globulus* essential oil then add 10 ml of 96% ethanol and put into formula 2, take 1 ml of *Citrus peel* essential oil limon and 1 ml of *Eucalyptus globulus* essential oil and then 10 ml of 96% ethanol in each solution, 5 mL of each solution was taken and added to formula 3. Added distilled water up to 100 mL and stirred until dissolved and a homogeneous gel was formed. Besides that formula, Dettol® and a placebo were also prepared. Dettol® acted as a positive control and placebo as negative control. The formula of hand sanitizer gel showed in table 1.

Tabel 1. Hand Sanitizer Gel formula

Materials	**F1**	**F2**	**F3**	**F4**
CLEO 10%	10 ml	-	5 ml	-
EGEO 10%	-	10 ml	5 ml	-
Carbopol 940	0.5 g	0.5 g	0.5 g	0.5 g
TEA	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Methyl Paraben	0.2 g	0.2 g	0.2 g	0.2 g
Glycerin	0.79 ml	0.79 ml	0.79 ml	0.79 ml
Ethanol 96%	15 ml	15 ml	15 ml	15 ml
Aquadest	Ad 100 ml	Ad 100 ml	Ad 100 ml	Ad 100 ml

Note:
CLEO : *Citrus limon* essential oil
EGEO : *Eucalyptus globulus* essential oil
F1 : 100% CLEO
F2 :100% EGEO
F3 : 50% CLEO; 50% EGEO
F4 : Placebo gel

Evaluation of Gel Hand sanitizer

Organoleptic

The organoleptic test was carried out by looking directly at the gel preparation for color, odor or aroma, gel form, easy to apply to the skin or hands, and no coarse granules (Widyawati et al., 2017).

Homogeneity

The homogeneity test of the preparation was carried out by smearing the preparation on two pieces of a watch glass or other transparent material. The homogeneity of the preparation was assessed from the absence of coarse grains. Observed using a microscope with a magnification of 40x (Nikam, 2017).

pH

The pH test was carried out by weighing the sample as much as 1 gram. 10 mL of distilled water pH 7 was added and then stirred. After being homogeneous, the pH of the preparation was measured by inserting a calibrated pH meter, allowed to stand for a while until a constant pH was obtained (Widyawati et al., 2017).

Spreadability

Spreadability test was carried out by taking 0.5 grams of gel and placing it in the middle of a petri dish with millimeter block paper attached to the bottom. Measured the spread of the gel with the diameter of the gel that spreads from two sides after being left for 1 minute. The diameter of the gel was measured without a load, a load of 50 grams, 100 grams, and 150 grams until a constant dispersion was obtained then after 1 minute the gel spread was recorded (Widyawati et al., 2017).

Adhesion Test

The adhesion test was carried out by placing 0.5 grams of gel on a slide and overwriting it with another slide, then observing it by giving a load of 1 kg for 3 minutes. Determination of adhesion in the form of the time required until the two slides are released (Yusuf et al., 2017).

Viscosity

Viscosity test was carried out using a viscometer and tested 100 mL of the gel preparation. Good gel viscosity of 50-1000 dPa.s, optimal 200 dPa.s (Pratiwi, Nurahmanto, et al., 2017).

Antibacterial Test

Prior to the antibacterial test, the tools and materials were sterilized using an autoclave at 121°C for 15 minutes. Nutrient agar media was made by dissolving nutrient agar powder with aquadest, and then heated using a magic stirrer until the nutrient agar solution became clear, and then bacterial stock and bacterial suspension were made. After that, an antibacterial test was obtained using the paper disc method.

Calculation of The Optimum Formula Composition

The value of pH, adhesion, inhibition zone of *Staphylococcus aureus*, *Escherichia coli* bacteria, inhibition zone of

Pseudomonas aeruginosa bacteria obtained were calculated using the *Simplex Lattice Design* equation as follows (Kurniawan et al., 2019):

$$Y = a(A) + b(B) + ab(A)(B)$$

Note

Y : test results
A : Citrus limon essential oil composition
B : Eucalyptus globulus essential oil composition
a : Citrus limon essential oil coefficient
b : Eucalyptus globulus essential oil coefficient
a,b : coefficient of essential oil of Citrus limon and essential oil of Eucalyptus globulus

Determination of the optimal formula was obtained from the largest total response based on the normalization calculation of the pH value, adhesion, inhibition zone of *Staphylococcus aureus* bacteria, inhibition zone of *Escherichia coli* bacteria, inhibition zone of *Pseudomonas aeruginosa* bacteria which had significant differences between formulas. Determination of the composition of the combination of *Citrus limon* essential oil and *Eucalyptus globulus* essential oil was obtained from the sum of the pH values (R1); adhesion (R2); zone of inhibition of *Staphylococcus aureus* (R3) bacteria; zone of inhibition of *Escherichia coli* bacteria (R4); zone of inhibition of the bacterium *Pseudomonas aeruginosa* with the weighting as follows:

R1 = N1 x 0.2; R2 = N2 x 0.2; R3 = N3 x 0.2; R4 = N4 x 0.2; R5 = N5 x 0.2

$$N = \frac{X - Xmin}{Xmax - Xmin}$$

X : the response of each test
Xmin : minimum response
Xmax : the maximum response

Then the total R value is calculated which is the result of the sum of the values R1, R2, R3, R4 and R5. The highest total R value is a hand sanitizer gel formulation with a combination of *Citrus limon* essential oil and *Eucalyptus globulus* essential oil with the best pH value, adhesion, inhibition zone for *Staphylococcus aureus* bacteria, *Escherichia coli* bacteria inhibition zone, and the best *Pseudomonas aeruginosa* inhibition zone.

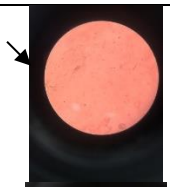
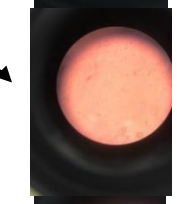
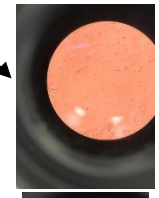
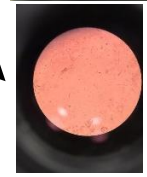
RESULTS AND DISCUSSION

Table 2. Organoleptic Test Results for Hand Sanitizer Gel

Formula	F1	F2	F3
Form	Gel	Gel	Gel
Odor	*Citrus limon* essential oil	*Eucalyptus globulus* essential oil	Combination between *Citrus limon* essential oil and *Eucalyptus globulus* essential oil
Color	Ivory	Ivory	Ivory

The results of the organoleptic test showed in table 2. The form of all the tested formulas was semi-solid gels. The gel form was due to the use of carbopol 940 as gelling agent that had high stability (Allen, 2002). The odor of each formulation was influenced by the essential oil as the active substance contained in the formulas. The addition of essential oils affected the color of the hand sanitizer gel.

Table 3. Results of Homogeneity Test of Gel Hand Sanitizer Preparations

Formula	Result		
	Visual	Microscope 40x	Microscope 100x
F1			
F2			
F3			

The organoleptic test results indicated that the tested formula visually did not show any coarse grains. However, when the formula was viewed through a microscope with a magnification of 40x and 100x, it could be seen that there were still coarse grains as showed by the arrow lines the table 3. The coarse grains might come from carbopol 940 which had not been completely crushed. To avoid the presence of these coarse grains, the formula can be made into a Nano emulsion or macro emulsion or mixing the carbopol 940 using Ultra Turrax at a certain speed.

Table 4. Result of pH test on Hand Sanitizer Gel

Formula	pH value
F1	5,07
F2	5,14
F3	5,01
+ Control	6,38
- Control	5,51

The test results on the pH values of each formula in this study showed that all the formulas tested were in the range of pH standard for skin, which is between 4.5 to 7 (Wijaya, 2013). The use of carbopol 940 as a gelling agent for hand sanitizer gel which is acidic was neutralized with TEA which is base as a neutralizing agent (Widyawati, 2017). A pH that is too acidic can cause irritation to the skin, while a pH that is too base can cause dry skin (Wijaya, 2013). The SLD chart shows that the pH values of the formulas that were tested were in the range of 5 to 6, which is a weak acid pH. This is due

to the nature of the essential oil itself which is composed of acidic and neutral compounds (Witri, 2017). The results of the pH statistical test between formulas tested with a 95% confidence level showed that F1 vs F2, F1 vs F3, F1 vs negative control, F2 vs F3, F2 vs negative control, F3 vs negative control had a p value> 0.05, which means no significantly different while F1, F2, F3 vs the positive control, namely Dettol®, had a p value of <0.05, which means that it was significantly different. Although there are significant differences between the formulas made, namely F1, F2, and F3 with Dettol® hand sanitizer, it does not have a big effect because the pH of all these preparations is in the pH range that corresponds to the skin's pH, which is 4.5-7 (Wijaya, 2013)

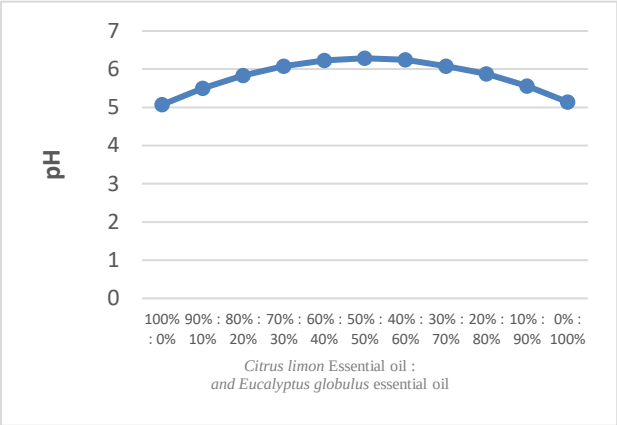


Figure 1. SLD Chart of CLEO and EGO Composition to pH value of Hand Sanitizer Gel

Table 5. Spreadability test result of Hand Sanitizer Gel

Formulas	Spreadability (cm)
F1	5,81±0,64
F2	6,12±0,61
F3	5,54±0,29
+Control	5,88±0,78
-Control	5,6±0,52

Rachmalia et. al. (2016) stated that a good spreadability of a topical formula is about 5-7 cm. The spreadability values of each formula that were tested in this study were in the standard range of spreadability standard value. Because of that, the hand sanitizer gel formulas are expected to have effective effects. The SLD chart shows that the proportion of 0% CLEO and 100% EGEO produced the highest spreadability among the hand sanitizer formulas that were tested, while the lowest spreadability was the formula with the proportion of 60% CLEO and 40% EGEO. The addition of essential oils will increase the spreadability of the hand sanitizer gel because the consistency becomes more watery. Spreadability is inversely related to viscosity and adhesion. The higher the spreadability, the lower the viscosity and adhesion and vice versa (Manasip et al., 2019). The results of the statistical test of dispersion between formulas with a 95% confidence level showed that F1 vs F2 vs F3 vs positive control vs negative control had a p value> 0.05, which means that there was no significant difference. The results of the dispersibility test using the *Simplex Lattice Design* show that the composition of *Citrus limon* essential oil and *Eucalyptus globulus*

essential oil affects the spreadability of the hand sanitizer gel preparation depending on the proportion used. Table 6. The Adhesion test result of Hand Sanitizer Gel

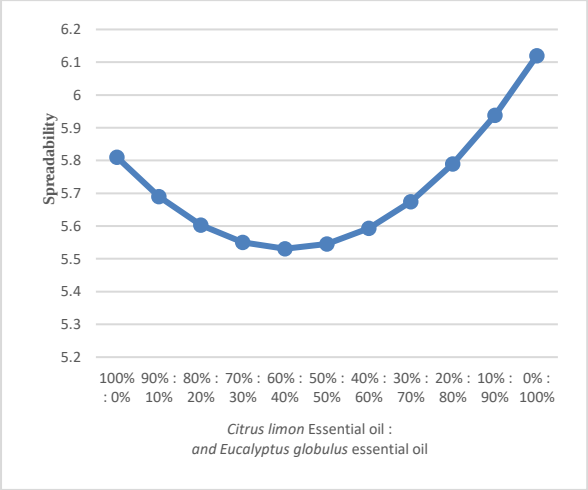


Figure 2. SLD Chart of CLEO and EGEO composition to the spreadability value of Hand Sanitizer Gel

The standard of adhesion is more than 1 second, which means that each hand sanitizer gel formulas tested are in the standard value of adhesion (Yusuf et al., 2017). The longer a formula stick in the surface, the better it is in giving effect (Hastuty et al., 2018). The SLD chart shows that at the proportion of 60% CLEO and 40% EGEO, it produced a longer adhesion of the hand sanitizer formula, while the fastest adhesion is predicted at the proportion of 0% CLEO and 100% EGEO. The stickiness of hand sanitizer gel is directly proportional to the viscosity and inversely proportional to the spreadability so that the more use of carbopol 940 as a gelling agent, the viscosity and time of adhesion will increase and vice versa (Manarisip et al., 2019). The results of the statistical test of stickiness with a 95% confidence level showed that F1 vs. F2 vs. F3 vs. positive control vs. negative control had a

Formula	Result (minute)
F1	7,3 ±2,64
F2	6,9±1,72
F3	8,1±3,12
+Control	6,5±2,19
- Control	6,8±1,24

p value of > 0.05, which means that it was not significantly different. The results of the dispersibility test using the *Simplex Lattice Design* show that the composition of *Citrus limon* essential oil and *Eucalyptus globulus* essential oil affects the adhesion value of the hand sanitizer gel preparation depending on the proportion used.

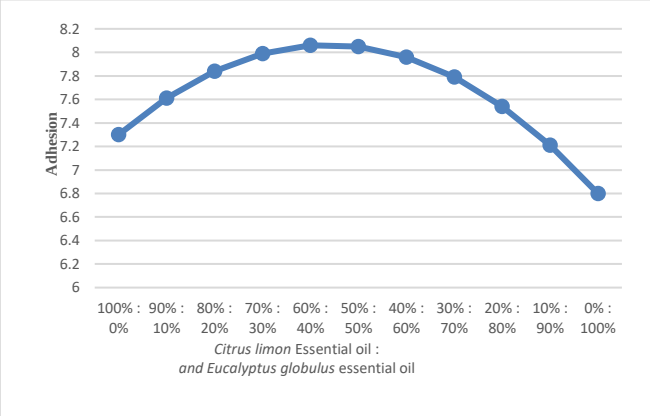


Figure 3. SLD Chart of CLEO and EGEO composition to the adhesion value of Hand Sanitizer Gel

Table 7. Viscosity Test Result of Hand Sanitizer Gel

Formula	Viscosity (dPa.s)
F1	171
F2	170
F3	171.3

A good gel formula has a standard viscosity value between 50 to 1000 dPa.s, while the optimal viscosity value is 200 dPa.s (Pratiwi, Nurahmanto, et al., 2017). The results of the viscosity testing of each hand sanitizer gel formula indicated that all formulas tested were in the range of standard viscosity. In this range, gel formula has a good consistency which affects the spreadability and will be easy to be taken out from the container when it's applied to the hands (Pratiwi, Nurahmanto., et al 2017).

Table 8. Results of Hand Sanitizer Gel Antibacterial Test

Formula	Test Result (mm)		
	S.aureus	***E.coli***	***P.aeruginosa***
F1	9.77±0.19	11.22±1.35	11.77±0.19
F2	10.55±0.50	12.77±1.17	11.88±0.38
F3	11.66±0.57	11.55±0.50	10.55±0.95
Control +	14.55±0.50	11.99±0.33	12.33±0.67
Control -	0	0	0

Note:
F1 : 100% CLEO
F2 : 100% EGEO
F3 : 50% CLEO ; 50% EGEO
Control + : Dettol®
Control - : Placebo

The results showed that the hand sanitizer gel with *Citrus limon* essential oil and Eucalyptus globulus essential oil had antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* bacteria, while placebo as a negative control had no antibacterial activity. Limonene as antibacterial works by damaging cell walls and damaging the DNA structure of bacteria. The damage of the cell wall and DNA structure causes a disturbance to the active transport and proton strength in the bacterial cytoplasmic membrane, resulting in denaturation and inactivation of proteins such as the 1,8-cineole enzyme which is a terpenoid group. Antibacterial activity occurs by reacting with the porin on the outer membrane of the bacterial cell wall

by forming a strong polymeric bond that causes damage to the porin. The porin damage will cause the infiltration of 1,8-cineole compounds and then reducing the permeability of the bacterial cell wall so that bacterial cells will lack nutrients, causing bacteria to be inhibited or die (Astiani et al., 2014). 5 ml of alcohol contained in the negative control (placebo) was not able to inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* bacteria. According to Asngad & Nopitasari (2018), a hand sanitizer can kill germs with an alcohol concentration of ± 60% to 80%.

The results of the antibacterial statistical tests of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, respectively, between the formulas tested with a 95% confidence level, showed that *Staphylococcus aureus* bacteria, namely F1 vs F2, F2 vs F3 and F2 vs positive control had p>0.05, which means not significantly different in inhibiting *Staphylococcus aureus* bacteria. *Escherichia coli* and *Pseudomonas aeruginosa* bacteria have the same interpretation of significance value data, namely F1 vs F2, F1 vs F3, F1 vs positive control, F2 vs F3, F2 vs control positive, F3 vs positive control had a p value> 0.05, which means that there was no significant difference in inhibiting *Escherichia coli* and *Pseudomonas aeruginosa* bacteria. Statistical test results for *Staphylococcus aureus* showed that F1 vs F3, F1 vs positive control, F1 vs negative control, F2 vs negative control, F3 vs positive control and F3 vs negative control had a p value <0.05, which means that they were significantly different in inhibiting bacteria. *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* bacteria it shows that F1 vs negative control, F2 vs negative control, F3 vs negative control has a p value <0.05 which means that it is significantly different in inhibiting *Escherichia coli* and bacteria.

F1 had a high inhibitory power against P. aeruginosa bacteria, a gram-negative bacteria, while F2 has a high inhibitory power against E. coli bacteria, which is also a gram-negative bacteria. Mardiana & Handayani (2016), stated that the thickness and thinness of the peptidoglycan layer in the cell wall affects the permeability of the cell wall, this causes the inhibition zone differences that formed between gram-positive and gram-negative bacteria. Gram-negative bacteria are composed of much less peptidoglycan while the peptidoglycan has lesser extensive cross-linking than gram-positive bacteria. This causes gram-positive bacteria to have lower permeability than gram-negative bacteria. That low permeability causes the active substance of the hand sanitizer gel difficult to penetrate the cell membrane of gram-positive bacteria and the inhibition zone formed is less than optimal. Disruption of peptidoglycan synthesis causes the cell wall to be

incompletely formed because it does not contain peptidoglycan so that the cell is only covered by a cell membrane which causes bacterial cells to die due to osmotic pressure which causes lysis.

F3 has great inhibition against S. aureus, a gram-positive bacterium. According to Muharni et al., (2017) S. aureus as a gram-positive bacterium has a relatively simple cell wall structure so that the antibacterial compounds contained in the hand sanitizer gel formula easily enter bacterial cells and find targets to work and form an inhibition zone. Candrasari et al. (2011), stated that the growth inhibition of the tested microbes was influenced by the concentration of the active substance which resulted its strong or weak ability to inhibit bacterial growth. According to Prihandani et al. (2015), increasing the concentration of the active substance will increase its antibacterial activity, but with the increased concentration of the active substance may not necessarily produce physical properties that meet the requirements (Hudaya et al., 2014). Septiani et al. (2017), stated that the inhibition zone formed was not always directly proportional to the increasing concentration of the active substance. The zone of inhibition can also be affected by the rate of diffusion of antibacterial compounds differences in the media in a certain time.

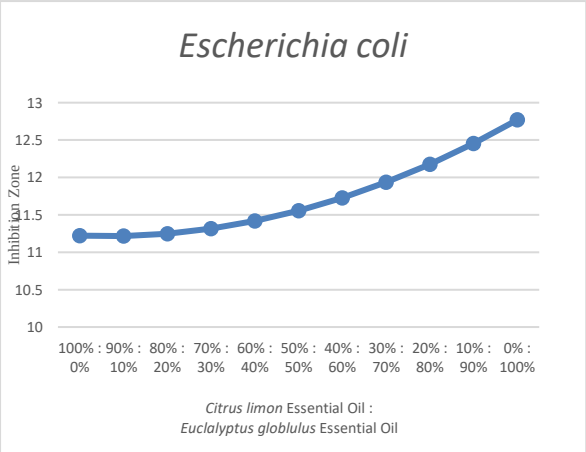


Figure 5. SLD Chart of Inhibition Zone Against *Escherichia coli*

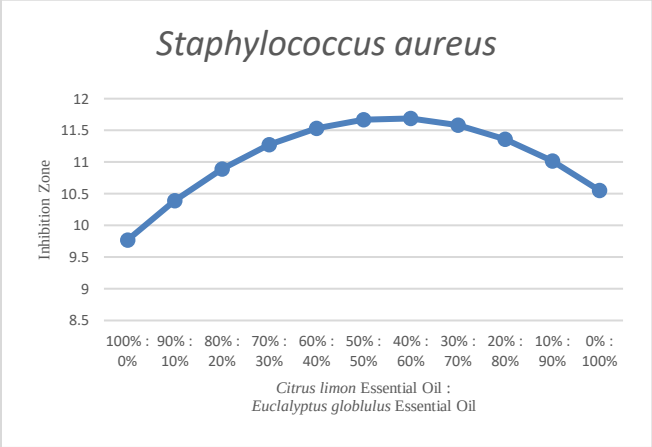


Figure 4. SLD Chart of Inhibition Zone Against *Staphylococcus aureus* Bacteria

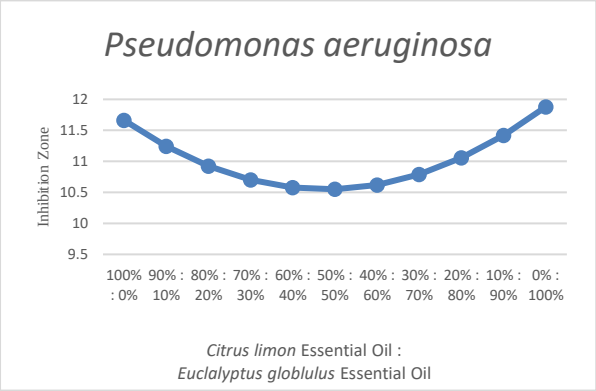


Figure 6. SLD Chart Inhibition Zone Against *Pseudomonas aeruginosa* Bacteria.

Determination of the optimum formula was carried out using the *Simplex Lattice Design* (SLD) method to obtain the formulation profile of the best combination of *Citrus limon* essential oil and *Eucalyptus globulus* essential oil. The parameters used in the SLD method for the inhibition zone are the results of the inhibition zone of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* bacteria and for physical properties, namely the results of the pH test and adhesion test. Not all test results of physical properties can be used as parameters in the use of SLD. The optimum composition used is to calculate the optimum total response obtained from the sum of the responses of the adhesion test and pH (Bolton & Bon, 2009). The choice of pH parameter is because pH is critical in a preparation because the pH of the preparation must be in accordance with the pH of the skin so that it does not cause irritation or dry skin when used (Wijaya, 2013). The selection of adhesion as a parameter used in SLD because high adhesion will provide more effectiveness (Hastuty et al., 2018). The optimum formula is seen from the results of the largest total response based on normalization calculations.

The results of the normalization calculation for each parameter were weighted with a comparison of the inhibition zone of *Staphylococcus aureus* bacteria (0.2) : *Escherichia coli* bacteria inhibition zone (0.2) : *Pseudomonas aeruginosa* bacteria inhibition zone (0.2) : pH (0.2) : adhesion (0.2). Based on the results of the calculation of the largest total response, the combination of *Citrus limon* essential oil and *Eucalyptus globulus* essential oil is in the ratio of 20% *Citrus limon* essential oil and 80% *Eucalyptus globulus* essential oil so that based on the results of these calculations the optimum formula for hand sanitizer gel preparation is a combination of *Citrus limon* essential oil and *Eucalyptus globulus* essential oil with the addition of *Citrus limon* essential oil (20%) and *Eucalyptus globulus* essential oil (80%).

Citrus limon essential oil or *Eucalyptus globulus* essential oil are equally effective in inhibiting bacterial growth because they are included in terpene compounds that have antibacterial activity, but the concentration of essential oil use greatly affects the inhibition zone formed due to the use of small concentrations of active substances in the preparation, of course forming an inhibition

zone. small and vice versa. In addition, the type of bacteria also affects the inhibition zone formed. Gram-positive bacteria are composed of 90% peptidoglycan layer and the rest is teichoic acid while gram-negative bacteria are composed of layers and the fat content is relatively larger (11-12%). This is what can cause gram-positive bacteria to be more easily damaged by bacterial compounds compared to gram-negative bacteria (Lingga et al., 2016). Based on the results of the total response calculation from hand sanitizer gel formulas with *Citrus limon* essential oil and *Eucalyptuls globulus* essential oil in this study, it showed that the largest total response was indicated by the hand sanitizer gel formula containing 20% CLEO and 80% EGEO. The formula that is considered optimal in this study is the formula that has the largest total response value and has value closest to 1. Thus, based on the calculation it is known that the optimal formula is the hand sanitizer gel contains 20% *Citrus limon* essential oil and 80% Eucalyptus globulus essential oil. As shown in Figure 7, the response value of the sanitizer gel with the proportion of 20% CLEO and 80% EGEO is 0.65707.

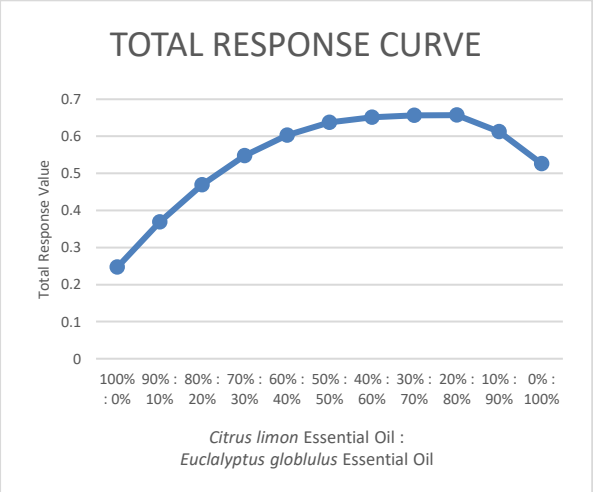


Figure 7. Chart of Relation Between *Citrus limon* Essential Oil and *Eucalyptus globulus* Essential Oil in The Hand Sanitizer Gel to the Total Responses.

The validation test of the *Simplex Lattice Design* (SLD) method was carried out to prove that the results of the formulation profile of the combination of *Citrus limon* essential oil and *Eucalyptus globulus* essential oil with the SLD method did not have a significant difference with the test results obtained from the optimum formula.

The validation test of the SLD method was carried out by testing the physical properties by looking at the parameters of pH and adhesion, and the antibacterial test by looking at the parameters of the inhibition zone of *Staphylococcus aureus* bacteria, the inhibition zone of *Escherichia coli* bacteria and the inhibition zone of *Pseudomonas aeruginosa* bacteria in the formula 20% *Citrus limon* essential oil and 80 % *Eucalyptus globulus essential* oil based on total response calculation. Comparison of test results between the calculated SLD data (theoretical) and the actual data (real) by using T-test analysis (Independent sample T-Test). The data table for the comparison of the results of the SLD analysis vs the experimental results is shown in Table 9 and the statistical analysis table for the validation of the SLD method is shown in Table 10.

Tabel 9. Data Comparison of SLD Theoretical Results vs Actual Results

Parameter	Inhibition Zone of Bacteria (mm)			pH	Adhesion (minutes)
	Staphylococcus aureus	***Escherichia coli***	***Pseudomonas aeruginosa***		
SLD Theoritical	11.36	12.17	11.05	5.88	7.54
Actual Results	19.99	12.22	11.44	5.50	7.20
Significance value	0.161	0.614	0.467	0.815	0.268
Data interpretation	Not significantly different	Not significantly different	Not significantly different	Not significantly different	Not significantly different

Table 10. Statistical Analysis of SLD Method Validation

Formula	Inhibition Zone of Bacteria (mm)			pH	Adhesion (minutes)
	Staphylococcus aureus	***Escherichia coli***	***Pseudomonas aeruginosa***		
Theoretical vs Actual	p>0,05	p>0,05	p>0,05	p>0,05	p>0,05

The result of statistical test shows that p>0.05 which means that there is no significant difference between the theoretical result (SLD) and the real result (test). From these results, it can be concluded that the *Simplex Lattice Design* method is a method with proven validation and can be used as an optimization method for a formula to obtain the best combination formulation profile.

CONCLUSION

Based on SLD, the optimal formula is the hand sanitizer gel formula containing 20% CLEO and 80% EGEO. The physical properties profiles of the hand sanitizer gel formula are pH 5.50; adhesion 7.20 minutes. The ability to inhibit *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* bacteria were 11.35 mm, 12.22 mm, and 11.44 mm. Further research with more various concentrations of the active substance is needed to obtain a larger inhibition zone against bacteria.

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